Ethephon, Ethylene and Abscission Physiology of *Camellia*.

A thesis presented in partial fulfilment of
the requirements for the degree of
Doctor of Philosophy
in
Horticultural Science
at
Massey University, Palmerston North,
New Zealand.

Allan Brian Woolf

1992

Massey University Library
Thesis Copyright Form

Title of thesis:

(1) (a) I give permission for my thesis to be made available to readers in Massey University Library under conditions determined by the Librarian.

(b) I do not wish my thesis to be made available to readers without my written consent for 16. months.

(2) (a) I agree that my thesis, or a copy, may be sent to another institution under conditions determined by the Librarian.

(b) I do not wish my thesis, or a copy, to be sent to another institution without my written consent for 16. months.

(3) (a) I agree that my thesis may be copied for Library use.

✓ (b) I do not wish my thesis to be copied for Library use for 12 months.

Signed [signature] WOOLF

Date 19/11/92

The copyright of this thesis belongs to the author. Readers must sign their name in the space below to show that they recognise this. They are asked to add their permanent address.

NAME AND ADDRESS DATE

ABSTRACT

Ethylene application to leaves and floral buds of *Camellia* resulted in abscission with a lag period, the duration of which was dependent on ethylene concentration and cultivar. During this period, cellulase activity doubled in leaf abscission zones, and when abscission commenced, activity increased more rapidly. However, no increase in cellulase activity was observed in floral bud abscission zones. Propylene application revealed that autocatalytic ethylene production increased in leaf abscission zones prior to and decreased after abscission. However, in the leaf blade, no change in endogenous ethylene production was measured, nor were any signs of leaf senescence observed. Application of silver thiosulphate (STS) completely inhibited leaf abscission and delayed and reduced floral bud abscission in response to applied ethylene. This pointed to a similar role for ethylene in both organs, but that the abscission process of floral buds occurred at a faster rate than that of leaves. Application of ethylene for differing durations to floral buds and leaves demonstrated that regardless of ethylene treatment duration, abscission ceased less than 24 hr after ethylene removal indicating that continuous ethylene exposure is required to promote abscission of *Camellia* organs.

Measurement of abscission rate (time to 50% abscission) in response to a range of ethylene concentrations determined that floral buds were more sensitive (that is; responded more rapidly to lower ethylene concentrations) than leaves. Ethylene-sensitivity was influenced by organ maturity. As floral buds matured from initiation to flower opening, the rate of ethylene-promoted abscission increased, indicating greater sensitivity. Leaves were most sensitive to ethylene directly after bud break and sensitivity decreased until 12 weeks after cessation of stem extension; after this time, sensitivity did not change significantly over the next 3 years.

Low temperatures reduced the ethylene-promoted abscission rate of both leaves and floral buds with an exponential relationship. Low temperatures increased the ethylene concentration required to saturate the abscission response. Endogenous ethylene production of *Camellia* leaves increased with higher temperatures and peaked at 20^{o} to 25^{o}C.

Since ethylene release from ethephon may be described in terms of concentration and duration of ethylene exposure, the effect of time, temperature, cultivar, organ type and organ maturity on organ abscission response to ethephon application could be explained in terms of the ethylene-promoted response.

The level of ethylene- and ethephon-promoted abscission were explained in terms of the interaction of ethylene concentration and duration of exposure with organ type, organ maturity and temperature which determined the level of abscission response. Three mechanisms were important in determining the response to ethylene; ethylene-sensitivity, and rate of reaction and reversibility of the abscission process. The rate of the abscission process was determined by ethylene concentration, temperature, organ type and maturity. Since abscission was reversible in *Camellia*, the duration of exposure interacted with the abscission rate to determine the extent of abscission in response to ethylene or ethephon application.

In conclusion, the greatly expanded understanding of the ethylene-promoted abscission process carried out in this study facilitates control (promotion or inhibition) of abscission in *Camellia*. This enhances the possibility for culture and transportation of high quality *Camellia* plants from New Zealand.

TABLE OF CONTENTS

Page

ACKNOWLEDGMENTS vii
LIST OF FIGURES ix
LIST OF TABLES xiv

CHAPTER 1
GENERAL INTRODUCTION. 1
1.1 TRANSPORTATION AND STORAGE OF LIVE *CAMELLIA* PLANTS 4
1.1.1 INTRODUCTION 4
1.1.2 PLANT MATERIAL AND HANDLING 4
1.1.3 THE TRANSPORTATION ENVIRONMENT 5
1.1.4 CONCLUSION 8
1.2 GROWTH AND FLOWERING IN *CAMELLIA* 9
1.2.1 INTRODUCTION 9
1.2.2 VEGETATIVE GROWTH 9
1.2.2.1 Vegetative Bud Break 9
1.2.2.2 Shoot Growth 12
1.2.3 FLOWERING 13
1.2.3.1 Floral Initiation 13
1.2.3.2 Floral Bud Development 14
1.2.3.3 Flower Opening 15
1.2.3 SUMMARY 15
1.3 ETHYLENE AND ABSCISSION PHYSIOLOGY 17
1.3.1 ETHYLENE ACTION 17
1.3.1.1 Ethylene Metabolism 18
1.3.1.2 Ethylene Metabolism and Action 18
1.3.1.3 Ethylene Binding 19
1.3.1.4 Ethylene Binding and Action 20
1.3.1.5 Ethylene Inhibitors 22
1.3.1.6 Ethylene Action Models 24
1.3.1.7 Transduction of the Ethylene Response 26
1.3.1.8 The Role of Ethylene Binding in Maturity, Environmental, and Hormonal. Effects of Ethylene Action 27

1.3.2 ETHYLENE AND ABSCISSION 30
1.3.2.1 The Abscission Process 30
1.3.2.2 Organ Type and the Abscission Process 32
1.3.3 THE ROLE OF CELLULASE IN ABSCISSION 34
1.3.4 ABSCISSION AND OTHER GROWTH SUBSTANCES 37
1.3.4.1 The Role of Auxin in Abscission 37
1.3.4.2 The Role of Gibberellins, Cytokinins and ABA in Abscission 38
1.3.5 THE ETHYLENE BIOSYNTHETIC PATHWAY 40
1.3.6 CLIMACTERIC AND NONCLIMACTERIC ETHYLENE PRODUCTION 41
1.3.7 ETHEPHON 42
1.3.8 DEFINITION OF SENSITIVITY AND RESPONSIVENESS TO ETHYLENE AND ETHEPHON 43
1.3.8.1 Ethylene-Sensitivity 44
1.3.8.2 Ethephon-Sensitivity 46
1.3.9 ASSUMPTIONS INVOLVED IN THE APPLICATION OF ETHYLENE CONCENTRATIONS AND MEASUREMENT OF ETHYLENE PRODUCTION 47

CHAPTER 2
SELECTIVE REMOVAL OF FLORAL BUDS FROM *CAMELLIA* WITH ETHEPHON.
I. INFLUENCE OF ETHEPHON CONCENTRATION, ORGAN TYPE AND TIME OF APPLICATION. 51
2.1 INTRODUCTION 51
2.2 OBJECTIVES 52
2.3 MATERIALS AND METHODS 52
2.4 RESULTS 54
2.5 DISCUSSION 59
2.6 CONCLUSION 60

CHAPTER 3
SELECTIVE REMOVAL OF FLORAL BUDS FROM *CAMELLIA* WITH ETHEPHON. II. INFLUENCE OF ETHEPHON CONCENTRATION, TEMPERATURE AND LEAF MATURITY. 61
3.1 INTRODUCTION 61
3.2 OBJECTIVES 64

3.3 MATERIALS AND METHODS 64
3.4 RESULTS 68
3.5 DISCUSSION 85
3.6 CONCLUSION 92

CHAPTER 4
ETHYLENE SENSITIVITY AS AFFECTED BY ORGAN TYPE, ORGAN MATURITY AND TEMPERATURE, AND ENDOGENOUS ETHYLENE PRODUCTION IN *CAMELLIA*. 94
4.1 INTRODUCTION 94
4.2 OBJECTIVES 99
4.3 MATERIALS AND METHODS 100
4.4 RESULTS 104
4.5 DISCUSSION 115
4.6 CONCLUSION 131

CHAPTER 5
THE ETHYLENE-PROMOTED ABSCISSION PROCESS IN *CAMELLIA* AND ITS REVERSIBILITY. 133
5.1 INTRODUCTION 133
5.2 OBJECTIVES 141
5.3 MATERIALS AND METHODS 142
5.4 RESULTS 146
5.5 DISCUSSION 154
5.6 CONCLUSION 169

CHAPTER 6
GENERAL DISCUSSION. 171

REFERENCES CITED. 190

ACKNOWLEDGEMENTS

I am indebted to my supervisors, Dr John Clemens and Dr Julie Plummer, who have provided continual encouragement, challenge and guidance during my PhD. I am very grateful for the cheerful giving of time, ideas and energy to train me in the art of scientific research. I could not have hoped for a better team.

My loving thanks also to Louise, who has helped and encouraged me as a friend, fiancee, and wife over the years of this thesis. I am particularly thankful for her forbearance during a wedding, (short) honeymoon, and the many uncertainties and pressures of the writeup.

I am grateful to the Camellia Society of New Zealand for its generous support and particularly Colonel T. Durrant for literature on the genus *Camellia*. Thanks is also due to the Camellia Memorial Trust for scholarships and interest in the work, and to its members, particularly Dr Rod Bieleski.

I wish to thank those who have helped me climb the research learning curve, particularly Dr Art Cameron, and thanks to Dr Edna Pesis and Dr Mike McManus for patient instruction in cellulase extraction and measurement. Thanks also to Dr Ross E. Lill of Crop and Food Research Institute of New Zealand Ltd, Levin (formally Levin Horticultural Research Center, MAF Levin) for providing unpublished results of trials into storage and ethylene response of *Camellia* and to Margaret A. Scott of Efford Experimental Horticulture Station, Lymington, Hampshire, England for information on flowering and culture of *Camellia*. I am also grateful for the use of the Growth Rooms of the former DSIR Climate Laboratories and the help of the staff there; Dr Ian Warrington, Joselyn, Len and particularly the understanding of Nicki Gardener (nee Seager). I am also in debt to Greg A. Lang (Louisiana State University, Dept of Horticulture, Baton Rouge, LA) for his stimulating and helpful discussion of the role of ethylene in *Olea* abscission.

The support of the staff of the Department of Plant Science, Massey University is gratefully acknowledged. Of these Prof. Errol Hewett Bruce Christie, Dr Nigel Banks, Pam, Hugh, Colin, David, Chris and Lois deserve special thanks. I would particularly like to thank Jonathan Dixon for his training and patience with use of all GC's.

I am also indebted to the Nursery Research Centre for use of facilities for the culture of stock plants and help of its great technicians Franz Ripphausen, Lisa Burton, Andrew Rodgers, and Chris Barnaby.

There are always a key group of people who support you through these times, are great friends and are those who have made it all possible in many and varied ways: Craig Mowatt, Fiona Cayzer, Philippa Thorley, Karen Snoad, Shauna Sylvester, Peter Jeffery, Robyn Symcock, Paul Austin and Millie (The Old Battle-axe) Tetlow.

To all of my flatmates of the "Warriors", "Goodies" and the one and only "Pooh Corner" flats for lots of great times and help in many many ways, I am very thankful guys. Many thanks also to Jeremy Compton who helped extensively with obtaining and sorting references. Also thanks to these people who have counted buds, washed bottles, ground up chemicals etc etc etc etc: Tina, Karen, Carolyn, Stuart, Melony, Liz, Elana, Rick, Howie, Lisa and Toni. I have particularly appreciated the support of my parents over the writeup period in Auckland.

I also acknowledge the technical support of my stereo, nutritional support of Griffins Gingernuts, and narcotic support of Bell Tea and Nescafe Classic Coffee.

I am eternally grateful to my Lord who got me into this project, has strengthened me through it and without whose encouragement I would certainly have ditched it for a less stressful, but much less exciting career.

This thesis is dedicated to my secondary school science teachers, Mr Dryburugh, Mr Khan and Mr Milne.

LIST OF FIGURES

Page

CHAPTER 1

Figure 1.1. Yearly growth cycle of *Camellia* and factors influencing vegetative and floral organ development. 10

Figure 1.2. Simplified hypothetical model of the mode of action of ethylene in plant cells. 25

Figure 1.3. Model of mechanism of action of ethylene inhibitors 2,5-NBD and Ag^{+}. 26

Figure 1.4. Balance model of abscission. 39

Figure 1.5. Days to 50% abscission of leaves or to complete abscission of fruit in response to 0.1, 1, 10 and 100 $ul \cdot liter^{-1}$ ethylene in *Citrus*. 46

Figure 1.6. Model of factors influencing the application of ethylene, measurement of ethylene production and ethylene concentration at the ethylene receptor. 49

CHAPTER 2

Figure 2.1. Abscission of plant organs from *Camellia* 'Anticipation' 32 days after application of ethephon on 1 June 1988 (winter). 56

Figure 2.2. Abscission of plant organs from *Camellia* 'Anticipation' 14 days after application of ethephon on 3 March (early autumn) and 14 April (mid autumn) 1989. 57

Figure 2.3. Abscission of plant organs from *Camellia* 'Donation' 14 days after application of ethephon on 3 March (early autumn) and 14 April (mid autumn) 1989. 57

Figure 2.4. Apex of stem of *Camellia* 'Anticipation' with vegetative bud damaged by application of 1000 to 2000 $ul \cdot liter^{-1}$ ethephon. 58

Figure 2.5. Abscission of 3 leaf maturities (3-month-, 1-year- and 2- to 3-year-old) from *Camellia* 'Anticipation' 32 days after application of ethephon on 1 June 1988 (winter). 58

CHAPTER 3

Figure 3.1. Effect of temperature and ethephon concentration on final abscission proportion (%) of floral buds of *Camellia* 'Anticipation'. Ethephon application carried out on 7 May 1991..... 74

Figure 3.2. Effect of temperature and ethephon concentration on final abscission proportion (%) of leaves of *Camellia* 'Anticipation'. Ethephon application carried out on 7 May 1991..... 75

Figure 3.3. Effect of temperature and ethephon concentration on final abscission proportion (%) of vegetative buds of *Camellia* 'Anticipation'. Ethephon application carried out on 7 May 1991..... 76

Figure 3.4. Figure 3.4. Effect of temperature and ethephon concentration on abscission rate (1/days to 50% final abscission) of floral buds of *Camellia* 'Anticipation'. Ethephon application carried out on 7 May 1991..... 77

Figure 3.5. Effect of temperature and ethephon concentration on abscission rate (1/days to 50% final abscission) of leaves of *Camellia* 'Anticipation'. Ethephon application carried out on 7 May 1991. 78

Figure 3.6. Effect of temperature and ethephon concentration on abscission rate (1/days to 50% final abscission) of vegetative buds of *Camellia* 'Anticipation'. Ethephon application carried out on 7 May 1991..... 79

Figure 3.7. Effect of temperature and ethephon concentration on difference between final abscission proportion (%) of floral buds and leaves of *Camellia* 'Anticipation'. Ethephon application carried out on 7 May 1991..... 80

Figure 3.8. Effect of temperature and ethephon concentration on difference between final abscission proportion (%) of floral buds and vegetative buds of *Camellia* 'Anticipation'. Ethephon application carried out on 7 May 1991..... 81

Figure 3.9. Effect of temperature and ethephon concentration on difference between abscission rate (1/days to 50% final abscission) of floral buds and leaves of *Camellia* 'Anticipation'. Ethephon application carried out on 7 May 1991..... 82

Figure 3.10. Effect of temperature and ethephon concentration on difference between abscission rate (1/days to 50% final abscission) of floral buds and vegetative buds of *Camellia* 'Anticipation'. Ethephon application carried out on 7 May 1991. 83

Figure 3.11. Effect of temperature on abscission rate of 3 organs (floral buds, vegetative buds and leaves) of *Camellia* 'Anticipation' after ethylene application (10 *u*l·liter^{-1}) on 7 May 1991. 84

Figure 3.12. Effect of ethephon concentration on final abscission proportion (%) of 4 leaf maturities of *Camellia* 'Anticipation' after ethephon application at 16.7^{o}C on 7 May 1991. 84

CHAPTER 4

Figure 4.1. Effect of ethylene concentration on abscission rate (time to 50% abscission) of floral buds and leaves of *Camellia* 'Donation'. (Experiment 4.1). 108

Figure 4.2. Effect of ethylene concentration on abscission rate (time to 50% abscission) of floral buds and leaves of *Camellia* 'Anticipation'. (Experiment 4.1). 108

Figure 4.3. Effect of two saturating ethylene concentrations and temperature on abscission rate (time to 50% abscission) of leaves of *Camellia* 'Donation'. (Experiment 4.3). 109

Figure 4.4. Effect of a saturating ethylene concentration and temperature on abscission rate (time to 50% abscission) of floral buds and leaves of *Camellia* 'Donation'. (Experiment 4.2 and 4.3). 109

Figure 4.5. Effect of ethylene concentration and temperature on abscission rate (time to 50% abscission) of floral buds of *Camellia* 'Donation'. (Experiment 4.2). 110

Figure 4.6. Effect of ethylene concentration and temperature on abscission rate (time to 50% abscission) of leaves of *Camellia* 'Donation'. (Experiment 4.3). 110

Figure 4.7. Effect of leaf and floral bud maturity (treated over a year) on rate (time to 50% abscission) of ethylene-promoted abscission (10 *u*l·liter^{-1}) of *Camellia* 'Anticipation'. (Experiment 4.4). 111

Figure 4.8. Effect of leaf and floral bud maturity (treated over a year) on rate (time to 50% abscission) of ethylene-promoted abscission (10 *u*l·liter^{-1} of *Camellia* 'Donation'. (Experiment 4.4). 111

Figure 4.9. Effect of leaf and floral bud maturity (treated simultaneously) on rate (time to 50% abscission) of ethylene-promoted abscission (10 *u*l·liter^{-1}) of *Camellia* 'Anticipation'. (Experiment 4.5). 112

Figure 4.10. Effect of leaf and floral bud maturity (treated simultaneously) on rate (time to 50% abscission) of ethylene-promoted abscission (10 *u*l·liter^{-1}) of *Camellia* 'Donation'. (Experiment 4.5). 112

Figure 4.11. Effect of leaf maturity and ethylene concentration on abscission rate (time to 50% abscission) of ethylene-promoted abscission of *Camellia* 'Anticipation'. (Experiment 4.6). 113

Figure 4.12. Effect of temperature on basal endogenous ethylene production of single-leaf stem explants of *Camellia* 'Brian'. (Experiment 4.7). 113

Figure 4.13. Arrhenius plot of temperature vs basal endogenous ethylene production of single-leaf stem explants of *Camellia* 'Brian'. (Experiment 4.7). 114

Figure 4.14. Effect of floral bud maturity (treated over a year) on rate (time to 50% abscission) of ethylene-promoted abscission (10 *u*l·liter^{-1}) and floral bud volume of *Camellia* 'Anticipation'. (Experiment 4.4). 114

Figure 4.15. Effect of cultural environment (green house (Experiment 4.4) or shade house (Experiment 4.5)) on abscission rate (time to 50% abscission) of floral buds of a given maturity (volume). Abscission promoted by ethylene (10 *u*l·liter^{-1}) of *Camellia* 'Anticipation'. (Experiment 4.4 and 4.5). 115

CHAPTER 5

Figure 5.1. Abscission proportion of floral buds and leaves of *Camellia* 'Anticipation' treated with 10 *u*l·liter^{-1} ethylene continuously (Experiment 5.1). 149

Figure 5.2. Abscission proportion of floral buds of *Camellia* 'Anticipation' treated with 10 *u*l·liter^{-1} ethylene for 18, 31, 42, 54, 74 hr, and continuously (Experiment 5.1). 150

Figure 5.3. Abscission proportion of leaves of *Camellia* 'Anticipation' treated with 10 *u*l·liter^{-1} ethylene for 18, 31, 42, 54, 74 hr, and continuously (Experiment 5.1). 150

Figure 5.4. Cellulase activity of 30 leaf abscission zones over the ethylene-promoted abscission process of *Camellia* 'Brian'. (Experiment 5.5). ... 151

Figure 5.5. Ethylene production of leaf-blade explants of *Camellia* 'Brian' and *Citrus limon* 'Meyer' treated with 5000 *u*l·liter^{-1} propylene and air (control). (Experiment 5.2). 151

Figure 5.6. Effect of duration of exposure on mean ethylene production and abscission in response to propylene (5000 *u*l·liter^{-1}) and air (control) treatment of single-leaf stem explants of *Camellia* 'Brian'. (Experiment 5.2). 152

Figure 5.7. Effect of duration of exposure to propylene on mean ethylene production around the time of abscission. Single-leaf stem explants of *Camellia* 'Brian' were exposed to 5000 *u*l·liter^{-1} propylene. (Experiment 5.2). 152

Figure 5.8. Abscission proportion of leaves of *Camellia* 'Anticipation' treated with silver thiosulphate (STS; 0.2 mmol·liter^{-1}, 24 hr) or double distilled water and subsequently exposed to 0, 1, or 10 *u*l·liter^{-1} ethylene. (Experiment 5.3). 153

Figure 5.9. Abscission proportion of floral buds of *Camellia* 'Anticipation' treated with silver thiosulphate (STS; 0.2 mmol·liter^{-1}, 24 hr) or double distilled water and subsequently exposed to 0, 1, or 10 *u*l·liter^{-1} ethylene. (Experiment 5.3). 153

LIST OF TABLES

Page

CHAPTER 2

Table 2.1. Summary of temperature and relative humidity for three ethephon application times. 56

CHAPTER 3

Table 3.1. Effect of temperature (10^{o} to 30^{o}C) on Q_{10} and absolute change in final abscission proportion of three *Camellia* organs treated with ethephon (0 to 4000 *u*l·liter^{-1}). 72

Table 3.2. Effect of temperature (10^{o} to 30^{o}C) on linear regressions produced ^{1}line slope, ^{2}slope SE, and ^{3}correlation coefficient (r^2) of final abscission proportion of three *Camellia* organs. 72

Table 3.3. E_a (kJ·mol^{-1}) derived from abscission rate (1/time to 50% final abscission) after ethephon (0 to 4000 *u*l·liter^{-1}) and ethylene (10 *u*l·liter^{-1}) treatment at temperatures of 10^{o} to 30^{o}C of three *Camellia* organs. 73

Table 3.4. Proportion of temperature effect on ethephon-promoted abscission rate due to influence of temperature on ethylene-promoted abscission rate. 73

CHAPTER 5

Table 5.1. Cellulase activity of abscission zones of floral buds and leaves of *Camellia* 'Brian' before ethylene application (control) and at completion of ethylene-promoted abscission (abscised). 149